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Development Of Pattern Recognition Techniques for the Evaluation of Toxicant Impacts to Multispecies Systems

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The program has evaluated the toxicity of two complex mixtures, the water soluable fractions (WSF) of the commercial turbine fuel Jet-A and the military fuel JP-4 using single species toxicity tests as well as the Standard Aquatic Microcosm (SAM). The WSF were not particularly toxic to the algal species tested although toxicity was observed when Daphnia magna was used as the test organism. The SAM experiments have been completed using concentrations of 0.0, 1, 5 and 15 percent WSF. Among the more interesting effects were the shifts in time of population peaks and some other variables compared to controls. Regression analysis of control to treatment groups often demonstrated only weak correlations. Multivariate nonmetric clustering (NMC) analysis, however, also demonstrated a marked separation between the 4 treatment groups for the Jet-A experiment. NMC proved to be the most powerful multivariate method of those examined for distinguishing the control and other treatment groups.

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19. ABSTRACT (Continued)

An additional research effort is focused on applying multivariate methods and other mathematical techniques into the process of ecological risk assessment. Application of multivariate methods coupled with new ways of distinguishing uncertainty have the potential for revolutionizing the risk assessment process.

Program Summary 91-92

The program has evaluated the toxicity of two complex mixtures, the water soluable fractions (WSF) of the commercial turbine fuel Jet-A and the military fuel JP-4 using single species toxicity tests as well as the Standard Aquatic Microcosm (SAM). The WSF were not particularly toxic to the algal species tested although toxicity was observed when *Daphnia magna* was used as the test organism. The SAM experiments have been completed using concentrations of 0.0, 1, 5 and 15 percent WSF. Among the more interesting effects were the shifts in time of population peaks and some other variables compared to controls. Regression analysis of control to treatment groups often demonstrated only weak correlations. Multivariate nonmetric clustering (NMC) analysis, however, also demonstrated a marked separation between the 4 treatment groups for the Jet-A experiment. NMC proved to be the most powerful multivariate method of those examined for distinguishing the control and other treatment groups.
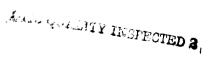
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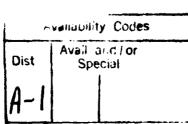
Program Objectives

The principal objective of this project is to examine the patterns in toxicity data from experiments using two microcosm protocols. We use nonmetric clustering, a multivariate pattern recognition technique developed by Matthews and Hearne (1991), for our primary pattern analyses. NMC has been shown to work well on a variety of ecological data sets (Matthews and Hearne, 1991; Matthews, et al., 1990a; Matthews, et al., 1990b). The results from the NMC analyses are then compared with those from other standard multivariate techniques to compare the utility of each technique for analyzing aquatic toxicity data.

Specific objectives are:

- Conduct one series of toxicity tests using the SAM and Mixed Flask Culture (MFC) protocols with 3 complex toxicants such as the water soluble fraction of JP-4, shale derived JP-4, and JP-8.

- For at least one of the complex toxicants, conduct a second complete series of toxicity tests (SAM and MFC) to compare similarities between parallel tests.

- Examine the SAM and MFC complex toxicant data using NMC, linear discriminant analysis, correspondence analysis, and metric clustering (k-means using Euclidean and cosine distances).

- Examine existing SAM data from experiments conducted previously for copper sulfate, brass, and graphite using NMC, linear discriminant analysis, correspondence analysis, and metric clustering.

- Describe a protocol that can be used for analyzing multispecies toxicity data. This protocol will incorporate a discussion of the advantages and limitations of the different multivariate analytical tools that were tested during this project.

Status of the Research

The results from the first year of the research program have been presented at the 1991 Annual Meeting of the Society for Environmental Toxicology and Chemistry (SETAC) in Seattle, and the recent Second Annual Symposium for Environmental Toxicology and Risk Assessment sponsored by Committee E47 of the American Society for Testing and Materials (ASTM) in Pittsburgh. In addition to the original research goals a direct fallout has been the application of the methodologies to environmental risk assessment. Several aspects of this research have a direct bearing upon the establishment of assessment endpoints, criteria for uncertainty determinations, and the overall risk assessment paradigm.

In year one the specific accomplishments met include:

- The evaluation of the toxicity of the water-soluble fractions of the fuels Jet-A and JP-4, both using standard algal and daphnid toxicity tests and the SAM.

- The examination of existing SAM data from experiments conducted previously for copper sulfate and the degradative bacterium *Alcaligenes dentirificans denitrificans* CR-1 using NMC, linear discriminant analysis, correspondence analysis, and metric clustering.

- Description of a protocol that can be used for analyzing multispecies toxicity data. This protocol incorporates a variety of methodologies and a discussion of the advantages and limitations of the different multivariate tools that were tested during this project has been presented at a recent meeting.

- Incorporation of the multivariate methodologies into a means of performing risk assessments at the community and ecosystems level. We are currently examining the utility of the methods derived from this research and related work into new methods of selecting risk assessment endpoints and for placing boundaries on uncertainty.

Below is a more detailed summary of our research program from June 1, 1991 to May 31, 1992.

Overview of the Methodology

Toxicants: Jet-A, JP-4 and JP-8 are the toxicants for these studies. The Jet-A has been obtained from a commercial supplier, Chevron. The military fuels have been obtained from the U.S. Air Force Laboratories at Wright-Patterson AFB and are labeled as to lot number. Records and archival samples are maintained by the Quality Assurance program of the Institute.

Microcosm Protocol: The 64 day SAM protocol as developed by Taub (Taub et al. 1988) consists of ten algal, four invertebrate and one bacterial species introduced into 3 L of sterile defined medium. Test containers are 4 L glass jars. An autoclaved sediment consisting of 200 g silica sand and

0.5 g of ground chitin are added to the already autoclaved jar and media. All complex toxicants are tested by removing 450 ml of media and organisms at the end of the 7 day acclimation period and adding appropriate amounts of jet fuel WSF and clean media that results in the final concentrations of toxicant. Concentrations for the tests run to date are 0, 1, 5 and 15 percent WSF. Numbers of organisms, dissolved oxygen (DO) and pH are determined twice weekly. Nutrients (nitrate, nitrite, ammonia, and phosphate) are sampled and measured twice weekly for the first four weeks, then only once weekly thereafter. A summary of the SAM methodology is presented in Table 1.

Sampling and Data Collection Procedures: All SAM data are recorded onto a Macintosh Classic, hard copy printed, checked for accuracy and archived. Then the information is then fed into the Macintosh compatible data analysis system (SAMS) developed by the University of Washington under contract with the Chemical Research Development and Engineering Center. Parameters calculated included the DO, DO gain and loss, nutrient concentrations, net photosynthesis/respiration ratio (P/R), pH, algal species diversity, daphnid fecundity, algal biovolume and biovolume of available algae. The statistical significance of each of these parameters compared to the controls are computed for each sampling day using the methodology of Conquest and Taub (1989).

Microcosm Research and Pattern Determination

Jet-A and JP-4 Standardized Aquatic Microcosms: Turbine fuels and particularly Jet-A are used throughout North America and are often the only aviation fuel available in most of the world. Large amounts are used by the military and airline carriers. As with most petroleum products, Jet-A consists of numerous constituents with varying water solubility, volatility and toxicity. Turbine fuels are in general much less volatile than gasoline.

This study investigates the toxicity of several preparations consisting of Jet-A and water using *Daphnia magna*, the green alga *Ankistrodesmus falcatus* and *Selenastrum capricornutum*, and the multispecies toxicity test the SAM.

Jet-A, refined by Chevron, was obtained from Fliteline Services Inc., Bellingham, WA. The JP-4 was kindly supplied by the U.S. Air Force Wright Patterson Laboratories. Two sets of formulations have been evaluated for toxicity in the acute tests. The first is prepared by vigorously mixing Jet-A and the SAM microcosm media in a separatory funnel, then waiting for the separation of the water and kerosene phases. The water phase, which contains the water-soluble fraction of the fuel, is collected, filtered to remove suspended material, and used for the first set of acute tests. The second method is identical to the first, but the resultant water phase is not filtered to remove the suspended globules of the fuel that have not yet entered solution.

Table 1. Summary of Test Conditions for a Typical Standardized Aquatic Microcosm
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ORGANISMS

Type and number of test
organisms per chamber:

<u>Algae </u>(added on Day 0 at initial concentration of 10^3 cells for each algae species):
Anabaena cylindrica, Ankistrodesmus sp.,*Chlamydomonas reinhardi* 90, *Chlorella vulgaris, Lyngbya* sp. *Nitzschia kutzigiana* (Diatom 216),*Scenedesmus obliquus, Selenastrum capricornutum, Stigeoclonium* sp., and *Ulothrix* sp.
<u>Animals </u>(added on Day 4 at the initial numbers indicated in parentheses):
Daphnia magna (16/microcosm),
Hyalella azteca (12/microcosm),
Cypridopsis sp. or *Cyprinotus* sp. (ostracod) (6/microcosm),
Hypotrichs [protozoa] (0.1/mL) (optional),
and *Philodina* sp. (rotifer) (0.03/mL)

EXPERIMENTAL DESIGN

Test vessel type and size:

One-gallon (3.8 L) glass jars are recommended; soft glass is satisfactory if new containers are used; measurements should be 16.0 cm wide at the shoulder, 25 cm tall with 10.6 cm openings

Medium volume:

500 mL added to each container

Number of replicates x concentrations   6x4

Reinoculation:

Once per week add one drop (circa 0.05 mL) to each microcosm from a mix of the ten species = 5×10^2 cells of each alga added per microcosm

Addition of test materials:

Add material on Day 7; test material may be added biweekly or weekly after sampling

Sampling frequency:

2 times each week until end of test

PHYSICAL AND CHEMICAL PARAMETERS

Temperature:

Incubator or temperature controlled room is required providing an environment 20 to 25°C with minimal dimensions of 2.6 by 0.85 by 0.8 m high

Light intensity:

80 μE m^{-2} photosynthetically active radiation s^{-1} (850 to 1000 fc)

Photoperiod:

12 h light / 12 h dark

Microcosm medium:

Medium T82MV adjusted to pH 7

Sediment:

Composed of silica sand (200 g), ground, crude chitin (0.5), and cellulose powder (0.5 g) added to each container

Typical Endpoints:

Population dynamics of each species, chemical-physical parameters, nutrients, diversity, predator-prey interactions, chemical fate.

Summary of Results to May 31, 1992

The short term toxicity tests resembled effluent tests in that the dilution series is set as a percentage as each preparation is examined. In the algal tests, low concentrations of Jet-A enhanced growth of the algae while the 100 percent concentration had no significant impact upon algal growth. Conversely, Jet-A is toxic to Daphnia. A dilution of 75 percent of the filtered Jet-A preparation is the 48 hr EC_{50} for *D. magna*. This dilution corresponds to approximately 4-6 mg/L concentration of organics. The unfiltered material EC_{50} for *D. magna* is much lower, on the order of 6-15 percent, although the amount of available toxics may be higher if the suspended material is also available to the Daphnia. Similar data were obtained for the WSF made from JP-4.

Two SAM experiments have been completed using concentrations of 0.0, 1, 5 and 15 percent WSF. The WSF is added on day 7 of the experiments by removing 450 ml from each microcosm, then adding the appropriate amount of toxicant solution and finally bringing the final volume to 3L with microcosm media. The effects of the WSF on the microcosm communities were subtle. Among the more interesting effects were the shifts in time of population peaks and some other variables compared to controls. Regression analysis of control to treatment groups often demonstrated only weak correlations. Multivariate nonmetric clustering analysis, however, also demonstrated a marked separation between the 4 treatment groups for the Jet-A experiment. The JP-4 experiments had similar results.

Application of multivariate techniques to the data analysis: One of the most challenging problems in aquatic toxicology is selecting statistical tests to be used on multivariate data sets and interpreting the results. We have compared three multivariate procedures to evaluate data from SAM tests. First, we used a similarity measure of samples within groups vs. samples from different groups, with a permutation test used to set significance levels. This test used the cosine of vectors distance metric as a similarity measure. In addition, we used a blind pattern analysis (clustering) of samples, invoking both k-means clustering with a Euclidean distance metric, and nonmetric clustering. The measure of association between the patterns in the data and the known treatment groups was used to set significance levels.

Our results from a 60-day SAM test involving the riot control chemical (CR) and a bacterium (CR-1) known to degrade CR are presented below. The data from SAM tests involving copper sulfate and brass dust are currently being evaluated.

The CR test had four treatment groups: control (group A), CR alone (group B), CR-1 alone (group C), and CR and CR-1 together (group D). Three replicate jars for each group were used, with two replicate samples from each jar, giving a total of 24 sample points for each sampling date. All three statistical techniques found no significant differences between A and C or between B and D; however, significant differences were found between A and B, A and D, C and B, and C and D, leading us to conclude that CR (or it's degradation products) remain toxic in the presence of CR-1. There were, however, large differences in the sensitivity of the three tests. Both the permutation test based on a

cosine metric and the clustering test based on a Euclidean metric indicated a significant response to the toxin beginning at about day 30 of the study and continuing to the end. Nonmetric clustering, on the other hand, indicated two distinct responses. The first response occurred on day 14, which corresponded to the primary effects of the toxicant on Daphnia (die-off in B and D). The second response occurred on day 30, which corresponded to secondary effects of the toxin (enhanced algal blooms in the absence of Daphnia). All three statistical approaches were valuable data analysis tools, but the nonmetric approach was the most sensitive to the subtle changes in this data set.

Replicability of the Standardized Aquatic Microcosm Protocol: We examined the replicability of the SAM protocol by pooling the control group data from several SAM experiments conducted in different labs and at different sites. While it may be obvious to the eye that similar patterns occur in the control groups from different experiments, numeric comparisons between two experiments are difficult. Each control group, for example, will typically have blooms of the several algal species or Daphnia in the same relative order and relative size, but the exact date of occurrence of the blooms, and their exact numerical size, may differ from experiment to experiment. Using several multivariate tests, we measured the differences between control groups from several SAM experiments. We found that, except for the kinds of problems mentioned, there is good replicability between experiments. Some suggestions for "transforming" microcosm data, by shifting dates and adjusting measurement units, are also under development.

Application of artificial intelligence techniques in the evaluation of environmental toxicology data: Many techniques developed by computer scientists in the field of artificial intelligence (AI) are currently being used in industry, academia, and government applications as standard, state-of-the-art technology. These techniques have proven their value and validity in such fields as medicine, geology, agronomy, and astronomy time and again. Unfortunately, few of these methodologies have penetrated the toxicological and ecological fields. Earlier we presented NMC, an analysis tool for multispecies data based on nonmetric clustering, an artificial intelligence technique developed specifically to aid in the interpretation of complex ecological data sets. This technique uses AI search to find an appropriate and meaningful characterization of a multivariate system, in conceptual terms. After appropriately characterizing the system in this fashion, the relationship between this characterization of the system and the critical environmental variables (pollution, toxicity, etc.) can be quantitatively analyzed to aid in the assessment of the effects of the environment on the system.

Population effects and community dynamics in the SAM-modeling compared to historical data sets with F. B. Taub, School of Fisheries, University of Washington and J.H. Taub, Seattle: Over the developmental period of the SAM and with its use to examine the population and community level effects

of several toxicants there is an extensive database that has accumulated. This database includes
experiments with copper sulfate, brass, graphite fibers, riot control materials and their degradative
organisms, antibiotics, and recently a series of water soluble fractions of turbine fuels. In addition, a
model to describe the SAM system has been published and recently modified. The accumulated research
facilitates the overview of the types of impacts seen, the duration, and a listing of the types of changes to
which the SAM system is relatively insensitive.

In several instances the release of predation has caused population blooms among the algal populations.
Among the more interesting effects were the shifts in time of population peaks and some other variables
compared to controls. Analysis of variance and regression analyses have demonstrated shortcomings.
Multivariate nonmetric clustering analyses as developed by Matthews *et al.* provided marked separation
between the 4 treatment groups.

MICMOD simulation model outputs also indicate the importance of timing of the stress; these
results will be presented in a related paper. Although generic in nature, the SAM system is useful at
looking at potential classes of community level effects.

Risk Assessment

Modification of the NAS risk assessment paradigm: Risk assessment paradigms for human
health have traditionally incorporated separate operational blocks for exposure and hazard assessment.
The results are transformed by an appropriate function into an estimate of risk. This methodology has
apparently worked well for estimates of risk to human health. In environmental risk assessment, a similar
analysis is inappropriate except in the case of an estimate of risk towards a single species.

Environmental risk assessments must take into account that once a xenobiotic is released to the
environment that the biological community alters the exposure and affects of the material. Biodegradation
and biotransformation also change the nature of the toxicant. In the process of this transformation the
toxicant often serves as a carbon source to segments of the community. The exposure and impacts of
non-chemical stressors also are modified in similar fashions. Current analysis suggests that the
traditional risk assessment paradigm be altered to create two different categories of environmental
exposure, prebiotic and biotic. Additionally, the hazard analysis needs to be broadened to include all
biological processes. Hazard to often includes only LD_{50} or MATC determinations separated from the
community dynamics of the biological system. Additionally, historical and evolutionary factors alter to
large degrees the response of a particular ecosystem to stress. In the prediction of community and
ecosystem level impacts it is essential that a multidimensional approach be incorporated. Lastly,
experimental or observational confirmation of the estimate of risk is crucial and should be an integral part
of the risk assessment process. Such verification can be utilized to improve estimation techniques and to
place realistic confidence intervals around determinations of risk.

Endpoints: Art or artifact? Much recent debate in toxicological studies has focused on appropriate endpoints for tests. We suggest that the search for endpoints appropriate to the entire field of toxicity testing is a fruitless academic search for a chimera that cannot be found. We recommend instead an approach that standardizes the common sense approach: different situations call for different endpoints. Typically, the toxicologist, if called upon for an expert opinion, will examine multivariate data, and extract from those data a few critical species. The behavior of these species will give an adequate (though perhaps not complete) picture of the toxic effects. Which species are selected, and whether it is their mortality, behavior, or biomass that is important, will vary from case to case. We call, therefore, for more research into the automation of the process typically performed by the expert. The selection of species, as well as other parameters, as significant for a particular experiment or field study, can be done automatically be computer algorithms. Many techniques developed in the field of machine learning, a specialized field in the general area of artificial intelligence, are currently being used in industry, academia, and government applications as standard, state-of-the-art technology. These techniques have proven their value and validity in such fields as medicine, geology, agronomy, and astronomy time and again, often beating the human experts at their own game. To be blind to the utility of these tools in the field of toxicology is to work by hand, over and over again, problems that could be solved in a twinkling with their aid.

Uncertainty propagation in risk assessment with J.W. Hearne, Computer Science Department, Western Washington University: Risk assessment typically proceeds by successively combining various uncertain inferences into an overall probability. For example, in computing the potential effect on a target species, an extrapolation may have to be made from an acute test on a similar species. A test on white mice, for example, may be pressed into service to estimate effects on deer mice. The expected exposure may be chronic rather than acute, and this will introduce further uncertainty. The test may have been an LC_{50} test, while the criteria standards may involve NOELs, which again have to be uncertainly estimated from the LC_{50}. Typically these uncertainties are combined into a single inferential step, often by assuming worst case in each step, and independence of each uncertainty. This procedure results in a conservative estimate, but rarely an accurate one. Further, it can create an unwarranted variance of several orders of magnitude from the actual test results. This type of inference procedure constitutes a probabilistic reasoning system, for which a number of mathematical formalisms have been developed in the artificial intelligence tradition, such as Dempster-Shafer theory, truth maintenance systems, and nonmonotonic logic. We use several cases to illustrate the differences between the conventional approach and more sophisticated approach that takes into account possible interactions between the various uncertainties in the system. It is generally possible to get much more realistic bounds on the risk assessment by invoking mathematical methods more sensitive to the logic of combined probabilities.

<u>Incorporation of multivariate techniques to the performance of ecological risk assessments in</u>
<u>cooperation with Anne Sergeant, ORD, U.S. EPA</u>: Unlike single species based risk assessments, it is often crucial in environmental or ecological risk assessments to be able to describe a system with many interacting components. These components do not fit the traditional risk assessment paradigm as exposure of the various biological components to the toxicant is altered by other parts of the same biological community. In addition, some quantifiable description of how different biological communities are upon the addition of a toxicant or some other stressor is required to describe adequately risk at the ecosystem level. Three methods have been applied at the ecosystem level, the mean strain measurement used by K. Kersting (1984, 1985, 1988) to describe impacts within a three compartment microcosm system, the state space analysis pioneered by A.R. Johnson (1988) used to evaluate data from the Giddings (1984) synfuel microcosms, and the nonmetric clustering developed by G. Matthews and R. Matthews (Matthews and Hearne, 1991, Matthews, Matthews and Ehinger 1991) for ecological datasets and for analysis of SAM data. Each method has direct application to the description of an effected ecosystem without reliance upon a single, specific, and perhaps misleading endpoint. Each also can assign distance or probability measures in order to compare the control to treatment groups. Nonmetric clustering (NMC) has the advantage of not attempting to combine different types of scales or metrics during the multivariate analysis and is robust against interference by random variables. Interpretation of effects, however, may be easier with the conventional metric methods. Application of these methodologies into an ecological risk assessment should have the benefit of combining large interactive datasets into distinct measures to be used as a measure of risk and as a test of the prediction of risk.

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Landis, W.G. Environmental risk assessment framework incorporating ecosystem level factors. Annual Meeting of the Society of Environmental Toxicology and Chemistry, Seattle, WA, November 5, 1991.

Landis, W.G., G.B. Matthews, R.A. Matthews and N.J. Shough. Evaluation of the Aquatic Toxicity of the Turbine Fuel Jet-A Using Single Species and Microcosm Toxicity Tests. ASTM Environmental Toxicology and Risk Assessment Symposium, Pittsburgh, PA, April 27, 1992.

G.B. Matthews, R.A. Matthews and W.G. Landis. Multivariate Analyses of Data from Aquatic Toxicity Microcosm Studies: a Comparison of Three Statistical Tests. ASTM Environmental Toxicology and Risk Assessment Symposium, Pittsburgh, PA, April 27, 1992.

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Landis, W.G., G.B. Matthews, R.A. Matthews, A. J. Markiewicz and N.J. Shough. Evaluation of the Aquatic Toxicity of the Turbine Fuel Jet-A Using Single Species and Microcosm Toxicity Tests. Annual Meeting, Pacific Northwest Chapter of the Society for Environmental Toxicology and Chemistry, Bellingham, WA, June 27, 1992.

G.B. Matthews, R.A. Matthews, and W.G. Landis. Multivariate Analyses of Data from Aquatic Toxicity Microcosm Studies: a Comparison of Three Statistical Tests. Annual Meeting, Pacific Northwest Chapter of the Society for Environmental Toxicology and Chemistry, Bellingham, WA, June 27, 1992.

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Landis, W.G., R.V. Sahakian, F.B. Taub and J.H. Taub. Population effects and community dynamics in the standardized aquatic microcosm-modeling compared to historical data sets. Annual Meeting Society of Environmental Toxicology and Chemistry, November 1992, Cincinnati, OH.

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Matthews, G.B., W.G. Landis and R.A. Matthews. Replicability of the control group response in the SAM. Annual Meeting Society of Environmental Toxicology and Chemistry, November 1992, Cincinnati, OH.

Landis, W.G. and A. Sergeant. Incorporation of multivariate techniques to the performance of ecological risk assessments. Annual Meeting Society of Environmental Toxicology and Chemistry, November 1992, Cincinnati, OH.

Landis, W.G., R.A. Matthews and G.B. Matthews. Endpoints: Art or artifact? Annual Meeting Society of Environmental Toxicology and Chemistry, November 1992, Cincinnati, OH.

Matthews, G.B., W.G. Landis and R.A. Matthews. Nonmetric clustering and association analysis: an artificial intelligence approach to multispecies data. Annual Meeting Society of Environmental Toxicology and Chemistry, November 1992, Cincinnati, OH.

Hearne, J.W. and G.B. Matthews. Uncertainty propagation in risk assessment. Annual Meeting Society of Environmental Toxicology and Chemistry, November 1992, Cincinnati, OH.

Papers in Preparation

Landis, W.G. , R.A. Matthews, A.J. Markiewicz, N.J. Shough and G.B. Matthews. Evaluation of the community level effects of the water soluble fraction of Jet-A using conventional and multivariate techniques. In preparation for *Environmental Toxicology and Chemistry* , submittal July 1992.

Matthews, G.B., W.G. Landis and R.A. Matthews. Non-metric clustering as a powerful tool for the evaluation of impacts in microcosm toxicity tests. In preparation for *Science* , submittal August 1992.

Landis, W. G. , A. J. Markiewicz, G. B. Matthews and R. A. Matthews. Comparison of the community level effects of Jet-A and JP-4 using multivariate techniques. In preparation for *Ecotoxicology*, submittal October 1992.

Students Supported by the Grant and Student Research Projects

Keel, Lester - Anthopluera as a Monitor for the Environmental Impacts of Toxicants (Dr. Landis-Huxley College).

Rodgers, Sara-Comparison of MFC toxicity tests with and without adapted communities (Dr. Landis-Huxley College).

Sahaklan, Robert - Population Dynamics and the Effects of Toxicants on Community Structure (Dr. Landis-Huxley College).

Roze, Michael. Application of RIFFLE program for data evaluation (Dr. G. Matthews-Computer Science).

Markiewicz, April J. Fate of Jet Fuel Water Soluble Fraction in the Standardized Aquatic Microcosm (Dr. R. Matthews-Huxley College)

Professional Collaborators in the Research Program

James W. Hearne, Computer Science Department, Western Washington University

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Interactions and Consultations

Over the last year this research has been translated into technology transfers to DOD and EPA laboratories and the private sector. Apart from presenting the research at national and international meetings, we have been successful in transferring this data and technology during informal meetings or presentations on-site. Below is a list of several of the groups with which we met of transferred information over the last 12 months.

Joseph Dulka, Agricultural Product Department, DuPont Experimental Station, Wilmington, DE. Microcosm use and data analysis.

Lidia Watrud, Team Leader, and Ray Siedler Biotechnology Team, U.S. EPA-Corvallis, OR. Data analysis from terrestrial microcosms.

Richard Bennett and Anne Fairbrother, Ecotoxicology Team, U.S. EPA Corvallis, OR. Data analysis of terrestrial field data.

Mark V. Haley and H. Dupont Durst, Chemical Research Development and Engineering Center, Aberdeen Proving Grounds, MD. Microcosm data analysis and result summary.

Nigel Blakley, Department of Ecology, Olympia, WA. Toxicity evaluation of petroleum mixtures.

SETAC Microcosm Workshop. Design and data analysis of microcosms for pesticide evaluations.

ICI Americas. Data analysis of aquatic microcosm studies.

Anne Sergeant, ORD, U.S. EPA., Washington, D.C. Application of multivariate methods to ecological risk assessments.